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CLINICAL STUDIES

Impaired Endothelium-Dependent Cholinergic Coronary Vasodilation In Patients With Angina and Normal Coronary Arteriograms

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The coronary vasomotor responses to selective infusion of graded concentrations (10^{-9} to 10^{-4} *M*) of acetylcholine into the left anterior descending artery were assessed by quantitative coronary arteriography in 24 patients with normal coronary arteriograms (12 patients with atypical symptoms and 12 patients with typical anginal pain) and 36 patients with coronary artery disease with different degrees of atherosclerosis of the left anterior descending artery.

In the patients with normal coronary arteries and atypical chest pain, acetylcholine induced predominantly a vasodilator response, which was maximal during a 10^{-5} *M* acetylcholine infusion. In contrast, in patients with coronary artery disease, acetylcholine caused dose-dependent vasoconstriction, which was observed even if the left anterior descending artery itself was smooth. Marked vasoconstriction was also induced in the patients with typical anginal pain and angiographically normal coronary arteries. In nine of these patients, this constrictor response was associated with anginal pain and electrocardiographic evidence of myocardial ischemia. Intracoronary administration of isosorbide dinitrate (1 mg) relieved the anginal pain and dilated all vessels.

These data suggest that 1) patients with normal coronary arteriograms and angina pectoris manifest impairment of endothelium-dependent vasodilation similar to that observed in patients with overt coronary atherosclerosis; and 2) abnormal coronary vasoconstrictor responses resulting from this impairment may contribute to the pathogenesis of myocardial ischemia and angina in these patients.

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Since the initial description of angina pectoris by Heberden, our understanding of the different pathogenetic mechanisms leading to myocardial ischemia and anginal pain has evolved significantly. The important role of a dynamic coronary artery stenosis leading to a transient decrease in coronary blood flow has been demonstrated in different subsets of patients with ischemic heart disease (1,2). However, the basic pathophysiologic mechanisms causing this abnormal vasoconstrictor activity of coronary arteries are not well known.

Recent advances in vascular physiology have demonstrated that the vascular endothelium has an important modulating effect on coronary artery tone through the release of one or more endothelium-derived relaxing factors (EDRF) (3–6). Experimental atherosclerosis leads to a major impairment of this relaxing factor-mediated endothelium-dependent vasodilation in peripheral and coronary arteries (7–15). The absence of a normally opposing endothelium-derived relaxing factor-mediated vasodilator effect further results in a potentiation of the vasoconstrictor effect of several vasoactive substances such as ergonovine and serotonin. These changes could explain the development of coronary spasm and lesser forms of a dynamic stenosis in diseased coronary arteries (16).

Studies (12,17) on isolated human coronary arteries have demonstrated that endothelium-dependent vasodilation is impaired in atherosclerotic vessels. Clinical studies (18,19) with selective intracoronary infusion of acetylcholine during coronary arteriography have shown that the normal endothelium-dependent vasodilator response is lost and that paradoxic vasoconstriction is observed in early and advanced atherosclerosis. An abnormal vasoconstrictor response to acetylcholine was also observed (20) in smooth coronary artery segments of patients with coronary artery disease evident in other vessels. Furthermore, an impairment of cholinergic endothelium-dependent vasodilation was observed (21,22) in patients with angiographically completely normal coronary arteries but with one or more coronary risk factors. The finding of completely smooth normal coronary arteries therefore does not preclude the presence of an impairment of endothelium-dependent vasodilation in the coronary circulation.

The present study examines the hypothesis that endothelium-dependent vasodilation could be impaired in patients with typical angina and angiographically normal coronary arteries. The presence of a dysfunction of endothelium-dependent vasodilation resulting in a lack of flow-mediated vasodilation and abnormal coronary vasoconstrictor activity was recently proposed (23,24) as a possible causal mecha-

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Table 1. Clinical Characteristics of 60 Patients

	Total No.	Age (yr)	Male Gender (no.; %)
Group 1: Normal coronary arteriogram	24		
1a: With atypical symptoms	12	47.7 ± 2.2	7/58
1b: With typical angina	12	51.2 ± 3.4	3/25*
Group 2: Coronary artery disease	36	55.8 ± 1.4†	28/78
2a: With a smooth left anterior descending artery	11	57.6 ± 2.6	9/82
2b: With a minor left anterior descending artery lesion (<30%)	13	53.1 ± 2.3	9/69
2c: With an advanced left anterior descending artery lesion (≥30%)	12	54.8 ± 2.8	10/83

*$p < 0.05$ versus patients with coronary artery disease (group 2); †$p < 0.05$ versus patients with normal coronary arteries and atypical chest pain (group 1a).

nism for the development of anginal pain and myocardial ischemia in such patients.

Methods

Study Patients

Coronary vascular responses to acetylcholine were assessed in 60 patients referred for diagnostic coronary arteriography. Patients with unstable angina, recent myocardial infarction (<6 weeks), clinical evidence of severe heart failure, severe left ventricular dysfunction or severe valvular disease were excluded from the study. The patients were classified into two main groups based on the presence or absence of coronary atherosclerosis as observed on the coronary arteriogram.

Group 1: patients with angiographically normal coronary arteries (Table 1). Twenty-four patients had entirely smooth coronary arteries on angiography. In all of these patients, left ventricular function was normal by ventriculography. This group of patients was further divided into two subgroups based on the presence or absence of typical anginal pain as recorded during the taking of the clinical history on the day before coronary arteriography was performed. Twelve patients with angiographically normal coronary arteries were classified as having atypical symptoms (group 1a). In a second group of 12 patients, the symptoms were considered to represent typical anginal chest pain (group 1b).

Classification of chest pain symptoms was performed by two independent and experienced cardiologists. Typical angina was defined as retrosternal pain described as tightness, pressure or constricting pain with or without typical radiation to the left arm, shoulder or jaw, occurring during exercise or at rest, or both, and typically relieved by rest or nitrates within 15 min.

Results of bicycle exercise testing were negative in 10 patients of group 1a, but revealed asymptomatic ST segment depression (>1 mm) on the electrocardiogram (ECG) during exercise in two patients. The latter two patients were not excluded from group 1a because their symptoms were very atypical for angina and acetylcholine infusion did not induce a marked vasoconstrictor response. In group 1b, results of exercise testing were abnormal in nine patients (chest pain associated with >1-mm horizontal or downsloping ST segment depression in five patients, fatigue and significant ST segment depression in four patients). In two patients, the test was submaximal because of early fatigue during exercise. One patient had a negative exercise test, but ambulatory Holter monitoring showed transient ST segment changes >2 mm at the moment of anginal pain.

Group 2: patients with coronary artery disease (Table 1). Thirty-six patients were classified as having coronary artery disease on the basis of coronary arteriographic findings (>50% diameter narrowing in at least one major coronary artery). All of these patients had stable angina pectoris, were in Canadian Cardiovascular Society class 2 or 3 and had no clinical history of variant angina. This group of patients was further subdivided into three subgroups depending on the presence and degree of atherosclerosis in the left anterior descending coronary artery: group 2a, 11 patients with an angiographically smooth left anterior descending artery without lumen irregularities; group 2b, 13 patients with a minor left anterior descending artery lesion ranging from lumen irregularities to <30% lumen diameter narrowing in the left anterior descending artery; and group 2c, 12 patients with a left anterior descending artery lesion with >30% lumen diameter narrowing.

Informed consent was obtained from all patients and the study protocol was approved by the Ethical Committee of the University Hospital of Antwerp.

Study Protocol

All vasoactive medication was withheld for ≥14 h before cardiac catheterization. Left heart catheterization, left ventricular angiography and coronary arteriography were performed with standard techniques by a percutaneous femoral approach. After completion of the diagnostic study, a 5F pacing catheter was advanced into the right atrium and atrial

pacing was started in the demand mode at a rate slightly higher than the basal sinus rate (+80 beats/min). A 7.5 or 8F left Judkins angioplasty guiding catheter was introduced into the left main coronary artery. Through this guiding catheter, a 3F coronary infusion catheter (Schneider, Medintag) was advanced over a 0.014-in. (0.036-cm) angioplasty guide wire into the proximal left anterior descending artery for selective intracoronary infusion of acetylcholine. Serial infusions of acetylcholine solutions with stepwise increasing concentrations (10^{-6}, 10^{-5} and finally 10^{-4} M) were performed during a period of 2 min using a peristaltic pump at a constant infusion rate of 2 ml/min, which constitutes ±2% of the estimated left anterior descending artery flow (±100 ml/min).

At the end of the protocol (or earlier if marked vasoconstriction accompanied by angina or ischemic electrocardiographic [ECG] repolarization changes occurred), 1 mg of isosorbide dinitrate diluted in 5 ml of saline solution was injected into the left coronary artery through the guiding catheter. Throughout all procedures, aortic blood pressure and the 12-lead ECG were monitored continuously. Coronary angiograms were obtained under control conditions, at the end of each infusion of acetylcholine and 30 s after the intracoronary administration of isosorbide dinitrate.

Quantitative Coronary Arteriography

Single-plane digital coronary arteriography was performed with the use of power injection of nonionic contrast medium (Ultravist 370, Schering). The images were acquired in an ECG-triggered pulsed mode at 11 frames/cycle in a 512 × 512 × 8-bit matrix (DG300 GE CGR). The position of the patient table, the C-arm and the image intensifier were left unchanged throughout the study protocol to keep the radiologic magnification factor constant. Special care was taken to select a view in which there was minimal overlapping of the left anterior descending artery with other coronary artery vessels.

Automatic stenosis analysis program. Diameters of the mid and distal left anterior descending artery and mid left circumflex coronary artery were measured using the automatic stenosis analysis program of the DG300 digital system. Automatic vessel segment contour detection is performed by this program using densitometric analysis techniques similar to those applied by other investigators (25). This program can briefly be described as follows.

After magnification (×2) of the image with interpolation of the data, the coronary artery segment to be measured is defined by the operator by indicating two points in the vessel. The digital data are resampled along scanlines perpendicular to the global direction of the vessel as determined by the line connecting the two points indicated by the operator. After a smoothing of the digital data by a 5 × 1-median filter, a centerline within the vessel segment to be measured is determined by densitometric analysis along the scanlines. Subsequently, the right and left borders of the coronary artery are defined on the basis of the maximal value of a weighted function of the density profiles along the scanlines right and left from the centerline. With the use of the detected contour points, a geometric centerline is generated and is subsequently smoothed by the use of a moving average filter. Thereafter, the positions of the detected border points are calculated along lines perpendicular to the geometric centerline of the vessel. From these positions, the diameters of the vessel are measured in pixels. The same edge detection system is used to determine the average diameter of the angiography catheter. With the knowledge of the exact diameter of the catheter, the radiologic magnification factor is calculated and all diameter measurements are thereafter converted from pixels to millimeters. All measurements were performed on nonsubstracted images and after logarithmic conversion of the data to account for Lambert-Beer exponential X-ray absorption.

Validation. This technique was validated by analysis of diameter measurements on digital images of coronary artery phantoms. In a Plexiglas block, precision models of coronary arteries with diameters of 1, 1.5, 2, 2.5, 3, 3.5 and 4 mm were filled with contrast medium and imaged with the DG300 digital angiography system. The overall accuracy (average difference of the computed result with the true values) was 4.2 ± 2.7% (mean ± 1 SD, n = 35 measurements); the precision (pooled SD of the differences) was 4.2 ± 3.6%.

Statistical analysis. All data are presented as mean values ± SEM. Paired Student's *t* tests were used to compare intragroup absolute lumen diameters at baseline with absolute lumen diameters after infusion of acetylcholine and after injection of isosorbide dinitrate. Coronary artery segment responses were identified as dilation, constriction or no change, depending on the presence or absence of a statistically significant difference from the baseline diameter. Changes in coronary diameter after infusion of acetylcholine and the administration of isosorbide dinitrate are expressed as percent change of the baseline diameter. Statistical comparisons between the patient groups of the relative changes in coronary artery diameter were performed by a one-factor analysis of variance (ANOVA). If a significant value was found, Scheffé's F test for multiple comparisons was used to identify differences between the different patient groups.

The incidence of coronary risk factors was compared between groups with use of the chi-square test. In the patients with angiographically normal coronary arteries, the relation between serum cholesterol level, age and total number of risk factors was evaluated by univariate linear regression analysis. The total number of risk factors was calculated considering the following risk factors: serum cholesterol levels >200 mg/dl, male gender, smoking, arterial hypertension and positive family history of ischemic heart disease. For all tests, statistical significance was assumed when the null hypothesis could be rejected at the 0.05 probability (p) level.

Table 2. Risk Factors in 60 Patients

	Serum Cholesterol (mg/dl)	Smokers (no./%)	Positive Family History of IHD (no./%)	No. of Risk Factors
Group 1: Normal coronary arteriogram				
1a: With atypical symptoms	214 ± 21	8/67	7/67	3.2 ± 0.3
1b: With typical angina	232 ± 14	6/50	6/58	2.9 ± 0.3
Group 2: CAD	258 ± 9	16/44	14/39	3.7 ± 0.2
p value*	0.46	0.41	0.47	0.09

*p value comparing groups 1 and 2. CAD = coronary artery disease; IHD = ischemic heart disease.

Results

Patient characteristics and coronary risk factors (Tables 1 and 2). The mean age was comparable in the two subgroups of patients with angiographically normal coronary arteries (group 1a, atypical symptoms: 47.7 ± 2.2 years; group 1b, typical angina: 51.2 ± 3.4 years). The patients with coronary artery disease (group 2) were significantly older (55.8 ± 1.4 years). The patients in group 1b contained significantly more women than did the overall group of patients with coronary artery disease (group 2), in which men were predominant. The serum cholesterol level and the total number of risk factors were not significantly different between the patients in group 2 and those in group 1a or 1b. All other risk factors were relatively equally distributed among the three groups of patients and the observed differences were not significant (chi-square test, $p > 0.05$).

Systemic hemodynamic responses to acetylcholine. In this study, heart rate was controlled by atrial pacing. Subselective infusion of acetylcholine into the left anterior descending artery was used to avoid atrioventricular conduction abnormalities. Indeed, bradyarrhythmias were not observed during the administration of acetylcholine. Systemic hemodynamics remained unchanged in all groups of patients.

Coronary Epicardial Artery Diameter Responses to Intracoronary Acetylcholine and Isosorbide Dinitrate

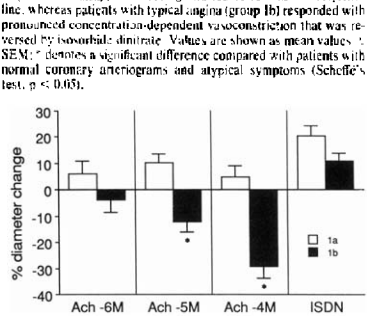
Effects of acetylcholine and isosorbide dinitrate. The diameter of the left circumflex artery, which was used as the control artery, showed minimal variability during the subsequent steps of acetylcholine infusion into the left anterior descending artery. The mean diameter change measured in all patients was −0.8 ± 0.6% (n = 153). Intracoronary injection of 1 mg of isosorbide dinitrate induced vasodilation of the circumflex artery. The increase in the circumflex artery diameter after isosorbide dinitrate was, respectively, 18.8 ± 3.3% ($p < 0.001$) in patients with normal coronary arteriograms and atypical symptoms (group 1a), 26.1 ± 3.9% ($p < 0.001$) in patients with normal coronary arteriograms and angina (group 1b) and 11.8 ± 2.5% ($p < 0.001$) in patients with coronary artery disease (group 2). The observed differences between groups were not significant (ANOVA, $p > 0.05$).

The diameter of the left anterior descending artery did not change significantly during infusion of 10^{-6} *M* of acetylcholine (Fig. 1). These data are therefore excluded from further discussion.

Patients with normal coronary arteriograms (group 1) (Fig. 1). In patients with angiographically normal coronary arteries, acetylcholine evoked no significant changes or mild vasodilation in patients with atypical symptoms (group 1a) but caused marked vasoconstriction in patients with typical angina (group 1b).

In the group of patients with no coronary artery disease visible on the coronary arteriogram and with atypical symptoms (group 1a). 10^{-5} *M* of acetylcholine induced mild vasodilation in the mid segment of the left anterior descending artery (10 ± 3.6%, $p < 0.01$) and a nonsignificant increase in the distal segment (4.4 ± 4.9%). After infusion of 10^{-4} *M* of acetylcholine this vasodilator response decreased and the diameters of the mid and distal segments of the left anterior descending artery were not significantly increased (4.9 ±

Figure 1. Vasomotor responses of the mid segment of the left anterior descending coronary artery to selective intracoronary infusion of graded concentrations (10^{-6} to 10^{-4} *M*) of acetylcholine (Ach) and the intracoronary injection of 1 mg of isosorbide dinitrate (ISDN) in patients with angiographically normal coronary arteries. In the patients with normal coronary arteriograms and atypical symptoms (group 1a), 10^{-5} *M* of acetylcholine evoked a vasodilator response that disappeared during infusion of 10^{-4} *M* of acetylcholine, whereas patients with typical angina (group 1b) responded with pronounced concentration-dependent vasoconstriction that was reversed by isosorbide dinitrate. Values are shown as mean values ± SEM; * denotes a significant difference compared with patients with normal coronary arteriograms and atypical symptoms (Scheffé's test, $p < 0.05$).



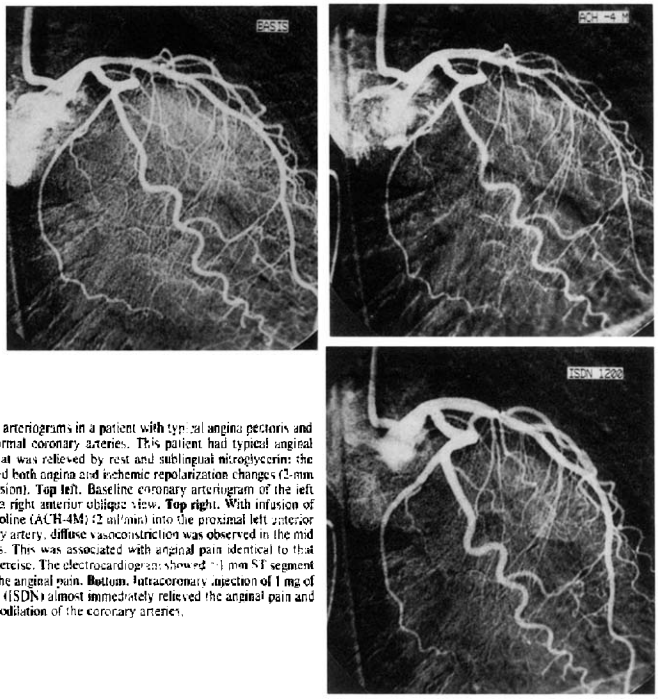


Figure 2. Coronary arteriograms in a patient with typical angina pectoris and angiographically normal coronary arteries. This patient had typical anginal pain on exertion that was relieved by rest and sublingual nitroglycerin; the exercise test induced both angina and ischemic repolarization changes (2-mm ST segment depression). **Top left.** Baseline coronary arteriogram of the left coronary artery in a right anterior oblique view. **Top right.** With infusion of 10^{-4} *M* of acetylcholine (ACH-4M) (2 ml/min) into the proximal left anterior descending coronary artery, diffuse vasoconstriction was observed in the mid and distal segments. This was associated with anginal pain identical to that occurring during exercise. The electrocardiogram showed 3-mm ST segment depression during the anginal pain. **Bottom.** Intracoronary injection of 1 mg of isosorbide dinitrate (ISDN) almost immediately relieved the anginal pain and induced diffuse vasodilation of the coronary arteries.

4.1% and 7.6 ± 6.3%, respectively). Intracoronary injection of isosorbide dinitrate evoked vasodilation of all segments. The relative diameter changes of the mid and distal left anterior descending artery after isosorbide dinitrate were, respectively, 20.2 ± 4% ($p < 0.001$ and 18.2 ± 4.1% ($p < 0.001$).

Paradoxically, in the patients with angiographically normal coronary arteries and typical angina (group 1b), infusion of 10^{-5} *M* of acetylcholine evoked vasoconstriction in the mid left anterior descending artery (−12.2 ± 3.9%, $p < 0.01$) and no significant change in the distal left anterior descending artery (−3.1 ± 2.4%). Infusion of 10^{-4} *M* of acetylcholine resulted in marked vasoconstriction of both the mid and distal left anterior descending artery. The mean diameter changes after 10^{-4} *M* of acetylcholine were, respectively, −29.2 ± 4.7% ($p < 0.001$) and −26 ± 3.9% ($p < 0.001$). An illustrative case is shown in Figure 2. Intracoronary administration of isosorbide dinitrate reversed this vasoconstriction and induced a diameter increase in the left anterior descending artery. The mean increases in lumen diameter of the mid and distal left anterior descending artery after isosorbide dinitrate were, respectively, 10.8 ± 2.9% ($p < 0.001$) and 12.1 ± 2.3% ($p < 0.001$).

Patients with coronary artery disease (group 2) (Fig. 3). In the 36 patients with coronary artery disease, acetylcholine induced dose-dependent vasoconstriction of the mid and distal segments of the left anterior descending artery. A discrete diameter decrease was observed in the mid and distal segments of this artery (−4 ± 1.8% and −3.9 ± 2.5%, respectively) in response to 10^{-5} *M* of acetylcholine. In some patients, the vasoconstrictor response induced by 10^{-5} *M* of acetylcholine was limited to the diseased left anterior descending artery segments. However, in most patients with a diseased left anterior descending artery,

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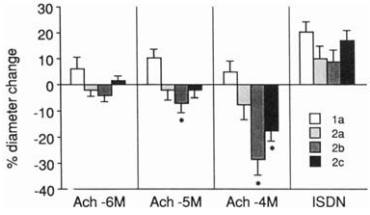

Figure 3. Vasomotor responses of the mid segment of the left anterior descending coronary artery to selective intracoronary infusion of graded concentrations (10^{-6} to 10^{-4} *M*) of acetylcholine (Ach) and intracoronary injection of 1 mg of isosorbide dinitrate (ISDN). Paradoxically, as compared with the response in patients with normal coronary arteries and atypical symptoms (group 1a), acetylcholine caused vasoconstriction in patients with coronary artery disease (group 2), which was most marked in patients with overt atherosclerotic lesions in the left anterior descending artery (groups 2b and 2c). Patients with coronary artery disease but with an angiographically smooth left anterior descending artery (group 2a) displayed an intermediate vasoconstrictor response to acetylcholine. Values are shown as mean values ± SEM; * denotes a significant difference compared with patients with normal coronary arteriograms and atypical symptoms (Scheffé's test, $p < 0.05$).

infusion of the highest concentration (10^{-4} *M*) of acetylcholine induced vasoconstriction in both the diseased and the adjacent angiographically normal coronary artery segments. The marked vasoconstriction occurred in both the mid and the distal left anterior descending artery segments. The mean changes in lumen diameter of the proximal and distal segments were, respectively, −19.0 ± 3.3% ($p < 0.001$) and −19.8 ± 4% ($p < 0.001$). Intracoronary administration of isosorbide dinitrate suppressed this vasoconstriction and induced a moderate diameter increase. The mean diameter changes in the mid and distal left anterior descending artery segments after isosorbide dinitrate were, respectively, 11.8 ± 2.4% ($p < 0.001$) and 12.6 ± 2.7% ($p < 0.001$).

In patients with coronary artery disease and a smooth left anterior descending artery (group 2a), the coronary artery diameter of the mid left anterior descending artery segments remained relatively unchanged after infusion of 10^{-5} *M* of acetylcholine (−2.2 ± 2.3%) and a nonsignificant diameter increase was observed in the distal segments (6.6 ± 5.9%). During infusion of 10^{-4} *M* of acetylcholine, a discrete diameter decrease was observed in both the mid and distal left anterior descending artery segments (−7.7 ± 5.7% and −4.5 ± 14.4%, respectively). Intracoronary administration of isosorbide dinitrate induced moderate vasodilation; the mean lumen diameter changes in the mid and distal left anterior descending artery segments were, respectively, 9.9 ± 3.4% ($p < 0.01$) and 17.1 ± 3.7% ($p < 0.01$).

In patients with coronary artery disease and a left anterior descending artery lesion with <30% lumen narrowing (group 2b), 10^{-5} *M* of acetylcholine elicited a mild diameter decrease in the mid and distal left anterior descending artery segments (−7.3 ± 3.7% and −5.9 ± 3.6%, respectively). Infusion of 10^{-4} *M* of acetylcholine induced marked vasoconstriction of the left anterior descending artery; the mean changes in the mid and distal segments were, respectively, −28.7 ± 6% ($p < 0.001$) and −24.6 ± 5.1% ($p < 0.001$). Isosorbide dinitrate reversed this vasoconstriction and induced vasodilation of the left anterior descending artery; the mean diameter changes in the mid and distal segments were, respectively, 8.6 ± 4.7% ($p > 0.05$) and 14.3 ± 6.5 ($p < 0.05$).

In patients with coronary artery disease and a left anterior descending artery lesion with >30% lumen narrowing (group 2c), 10^{-5} *M* of acetylcholine induced a nonsignificant diameter decrease in the mid and distal left anterior descending artery segments (−2.2 ± 2.8% and −7.8 ± 3.6%, respectively). Infusion of 10^{-4} *M* of acetylcholine elicited a marked vasoconstrictor response in the left anterior descending artery. The mean diameter changes in the mid and distal segments were, respectively, −17.8 ± 3.8% ($p < 0.01$) and −22.2 ± 6.1% ($p < 0.01$). These vasoconstrictor responses occurred not only at the site of the atherosclerotic lesions, but also in adjacent normal-appearing coronary artery segments after the infusion of 10^{-4} *M* of acetylcholine (Fig. 4). This vasoconstriction was rapidly inhibited by intracoronary administration of isosorbide dinitrate. The mean lumen diameter increases in the mid and distal left anterior descending artery segments were, respectively, 17 ± 3.9% ($p < 0.01$) and 8.5 ± 2.2% ($p < 0.01$).

Chest Pain

One patient in group 1a mentioned atypical chest pain without ECG changes during acetylcholine infusion, whereas all the other patients in this group remained asymptomatic. Nine patients in group 1b developed chest pain typical of angina pectoris during infusion of 10^{-4} *M* of acetylcholine. The pain symptoms in these patients were almost always identical in character and localization to those occurring spontaneously. Chest pain was accompanied by ECG evidence of myocardial ischemia (ST segment depression >1 mm) in five patients. Equivocal changes were observed in three patients (ST segment depression <1 mm in two patients and 1 mm of ST segment elevation in one) and one patient showed no ischemic repolarization changes during anginal pain. Fourteen patients with coronary artery disease (group 2) developed typical anginal pain during acetylcholine infusion (in 3 patients during infusion of 10^{-5} *M* of acetylcholine, in 12 patients during 10^{-4} *M* of acetylcholine). All patients in this group except one showed ECG signs of myocardial ischemia during the chest pain: ST segment depression >1 mm was observed in seven patients and ST segment elevation ≥2 mm in six others. Coronary arteriograms recorded during chest pain in the patients in group 1b and group 2 consistently showed marked coronary vasoconstriction in response to acetylcholine infusion. Intra-

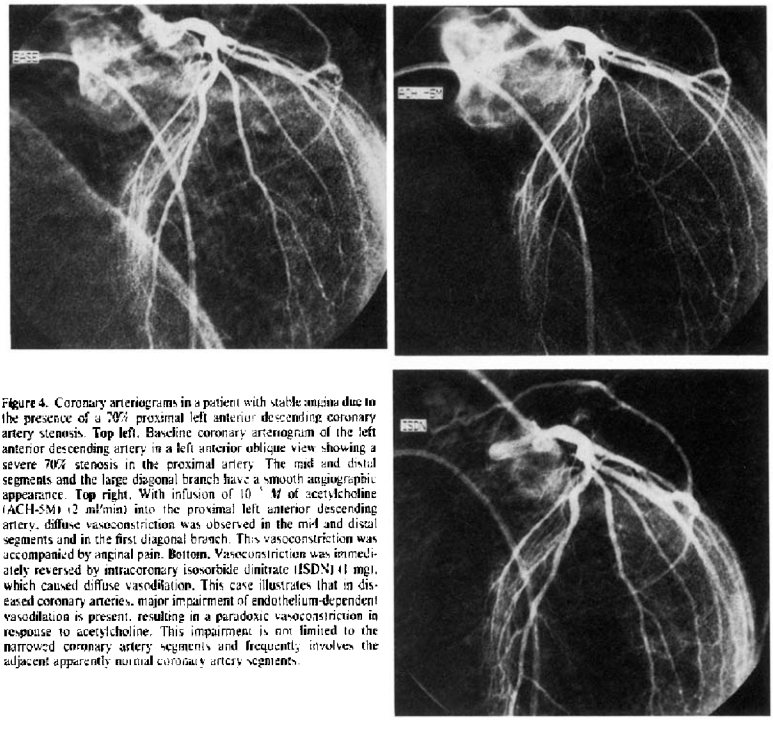

Figure 4. Coronary arteriograms in a patient with stable angina due to the presence of a 70% proximal left anterior descending coronary artery stenosis. **Top left.** Baseline coronary arteriogram of the left anterior descending artery in a left anterior oblique view showing a severe 70% stenosis in the proximal artery. The mid and distal segments and the large diagonal branch have a smooth angiographic appearance. **Top right.** With infusion of 10^{-4} *M* of acetylcholine (ACH-5M) (2 ml/min) into the proximal left anterior descending artery, diffuse vasoconstriction was observed in the mid and distal segments and in the first diagonal branch. This vasoconstriction was accompanied by anginal pain. **Bottom.** Vasoconstriction was immediately reversed by intracoronary isosorbide dinitrate (ISDN) (1 mg), which caused diffuse vasodilation. This case illustrates that in diseased coronary arteries, major impairment of endothelium-dependent vasodilation is present, resulting in a paradoxic vasoconstriction in response to acetylcholine. This impairment is not limited to the narrowed coronary artery segments and frequently involves the adjacent apparently normal coronary artery segments.

coronary administration of isosorbide dinitrate always caused reversal of the vasoconstriction, which resulted in rapid relief of the anginal pain and normalization of the ECG.

Relation Between Risk Factors and the Coronary Vasomotor Response to Acetylcholine

Among the patients with angiographically normal coronary arteries (group I), the vasomotor response to infusion of 10^{-4} *M* of acetylcholine showed no correlation with serum cholesterol levels ($r^2 = 0.001$, $p = 0.90$), age ($r^2 = 0.02$, $p = 0.56$) or number of risk factors present ($r^2 = 0.02$, $p = 0.49$) (Fig. 5). However, there was a clear relation with the presence and absence of typical anginal pain. All patients with typical anginal pain showed vasoconstriction with ≥10% lumen diameter narrowing on infusion of 10^{-4} *M* of acetylcholine, whereas most patients with atypical symptoms showed coronary vasodilation or a coronary artery diameter change <10% (Fig. 5). Only one patient with atypical chest pain showed coronary vasoconstriction with >10% lumen narrowing. However, this patient was >50 years of age.

Discussion

Effects of acetylcholine on normal coronary arteries in patients with angina. The major and original finding of the present study is that the normal endothelium-dependent vasodilator response to acetylcholine is lost in patients with angiographically normal coronary arteries and typical symptoms of angina pectoris to a degree similar to that observed in patients with overt coronary artery atherosclerosis. In most of the patients with angina and normal coronary

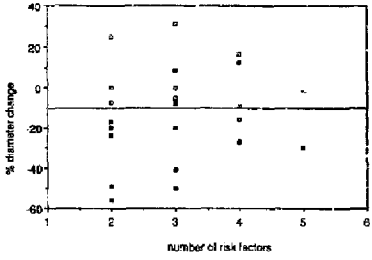

Figure 5. Plot of the relation between the vasomotor response to infusion of 10^{-4} *M* of acetylcholine and the total number of risk factors in patients with angiographically smooth coronary arteries. Univariate analysis did not demonstrate a significant correlation. In most patients with atypical symptoms (**open symbols**), a nonsignificant change or dilator response was observed, whereas patients with typical angina (**closed symbols**) generally showed constrictor responses.

arteries, the coronary vasoconstriction induced by acetylcholine was accompanied by the development of anginal chest pain and ECG signs of myocardial ischemia. The anginal pain was similar to that occurring spontaneously and intracoronary administration of isosorbide dinitrate rapidly reversed both the coronary vasoconstriction and the anginal pain. These observations suggest that abnormal coronary vasoconstrictor activity due to the presence of impaired endothelium-dependent vasodilation may contribute to the pathogenesis of myocardial ischemia in patients with angina and normal coronary arteriograms.

Studies in isolated arteries have demonstrated that the vascular endothelium has a potent modulating effect on coronary artery tone. Several vasoactive substances, including acetylcholine, induce endothelium-dependent vasodilation of normal coronary arteries by stimulating the endothelium to release one or more relaxing factors, causing relaxation of the vascular smooth muscle cells (3–6).

Acetylcholine has a dual effect on normal coronary arteries: endothelium-dependent vasodilation through the release of endothelium-derived relaxing factors and vasoconstriction by activation of muscarinic receptors on the vascular smooth muscle cells (3). If acetylcholine is infused into the coronary arteries, the net resulting coronary vasomotor response depends on the balance between these two opposing effects. Dose-response studies have demonstrated that endothelial stimulation occurs at lower acetylcholine concentrations than those required to produce contraction of smooth muscle. In normal coronary arteries with a functionally intact endothelium, acetylcholine will therefore induce vasodilation at low concentrations, whereas vasoconstriction is expected to occur at higher concentrations when the direct vasoconstrictor effect will override endothelium-dependent vasodilation. However, in diseased coronary arteries where the endothelium is dysfunctional as was shown by the in vitro studies, acetylcholine infusion is expected to induce vasoconstriction even at lower concentrations because normal endothelium-dependent vasodilation will no longer oppose the direct vasoconstrictor effect. By showing a reversal in the vasomotor response to intracoronary infusion of acetylcholine from vasodilation toward vasoconstriction at low concentrations or by demonstrating a potentiated vasoconstrictor response at higher concentrations, intracoronary infusion of acetylcholine may be a useful method for the in vivo assessment of endothelium-dependent vasodilation during coronary arteriography in patients.

Effects of acetylcholine on atherosclerotic arteries. The present study confirms previous clinical studies (18–20,26) with intracoronary or selective infusion of graded solutions of acetylcholine in the left anterior descending artery during coronary arteriography. As in those studies, acetylcholine in the present study induced mild coronary vasodilation or a nonsignificant change in patients with normal coronary arteries and paradoxic vasoconstriction in patients with coronary artery disease. The preservation of a vasodilator response to isosorbide dinitrate in all arteries suggests that the paradoxic vasoconstriction caused by acetylcholine in atherosclerotic coronary arteries is due to impairment of endothelium-dependent vasodilation. This dysfunction apparently occurs relatively early in the evolution of coronary atherosclerosis because an abnormal vasoconstrictor response to acetylcholine is already observed in arteries with minor disease. Moreover, in the patients with coronary artery disease, angiographically smooth coronary artery segments also showed a vasoconstrictor response to acetylcholine. It appears therefore that an impaired endothelium-dependent vasodilator response leading to unmasking of a potent vasoconstrictor response to acetylcholine is a very early manifestation of coronary atherosclerosis that frequently precedes the development of angiographically detectable coronary artery lesions.

It is of importance to note that this divergence of coronary vasomotor responses between patients with normal coronary arteries and atypical symptoms and those with coronary artery disease is observed at relatively low concentrations of acetylcholine. The vasodilator response to acetylcholine observed in patients with normal coronary arteries and atypical symptoms was maximal at an acetylcholine concentration of 10^{-5} *M* and disappeared in several patients of this group at a concentration of 10^{-4} *M*. This observation is in keeping with the different dose-responsiveness of the endothelium and vascular smooth muscle cells observed in in vitro studies (3) and in a recent study (26) in patients with normal coronary arteries and atypical chest pain in whom intracoronary infusion of up to 10^{-4}-*M* acetylcholine caused minor but progressive dilation of the left anterior descending artery, but constriction particularly of

the mid and distal artery segments occurred at higher concentrations (10^{-3} *M* and 10^{-2} *M*). The abnormal vasoconstrictor responses to acetylcholine observed in patients with coronary artery disease are therefore really paradoxic because they are caused by concentrations of acetylcholine 10- to 100-fold lower than those needed to induce vasoconstriction in normal coronary arteries.

Pathophysiology of abnormal vasoconstrictor response to acetylcholine. The major finding of the present study is the observation of an abnormal coronary vasoconstrictor response to acetylcholine in patients with angina and angiographically normal coronary arteries. Previous studies (27–32) have demonstrated that such patients have a functional disorder of the coronary circulation, with a reduced vasodilator response to pharmacologic stimuli (dipyridamole) and stress (rapid atrial pacing, exercise) and an increased vasoconstrictor response to ergonovine and cold pressor testing. Initially, it was thought that this abnormal vasomotor function was mainly localized at the level of the small precapillary intramyocardial arteries, but more recently it was demonstrated (33) that the epicardial coronary arteries also show an abnormal vasoconstrictor response during exercise. It is now widely accepted that these abnormal vasomotor responses are responsible for the development of myocardial ischemia and angina in these patients by causing a reduction in coronary flow reserve. However, the basic pathophysiologic mechanisms underlying this abnormal coronary vasomotion in patients with normal coronary arteries and angina, remained unknown. Recently, it was proposed (23) that impairment of endothelium-dependent vasodilation may contribute to the pathogenesis of angina and myocardial ischemia in patients with syndrome X. The present study supports this hypothesis by demonstrating paradoxic vasoconstrictor responses to acetylcholine in the group of patients with angina pectoris and angiographically normal coronary arteries (group Ib). Mild vasoconstrictor responses already occurred in these patients during infusion of 10^{-7} *M* of acetylcholine, which caused significant vasodilation in the patients with normal coronary arteries and atypical symptoms. Infusion of 10^{-4} *M* of acetylcholine elicited further coronary vasoconstriction as severe as that observed in the patients with overt coronary artery disease. The degree of coronary vasoconstriction was similar in the mid and distal segments of the left anterior coronary artery and marked vasoconstriction was also observed in the more distal and tertiary branches.

This paradoxic coronary vasoconstriction in response to acetylcholine and the presence of a preserved vasodilator response to isosorbide dinitrate in all constricting segments suggest that endothelium-dependent vasodilation is extensively impaired in the coronary circulation of patients with angina pectoris and normal coronary arteriograms. This impairment may result from either a primary endothelial cell dysfunction or the presence of microscopic atherosclerotic lesions not yet visible on the coronary arteriogram. The observation that most of the patients in group Ib developed anginal chest pain accompanied by ECG evidence of myocardial ischemia during the marked coronary vasoconstriction induced by acetylcholine further indicates that this impairment of coronary endothelium-dependent vasodilation may be a causal factor in the development of angina and myocardial ischemia in patients with angina and normal coronary arteriograms. Myocardial ischemia in these patients may result either from an inappropriate increase in coronary flow during exercise as a result of inhibition of endothelium-dependent flow-mediated vasodilation or from a transient decrease in coronary flow caused by augmented coronary vasoconstrictor responses that are no longer opposed by the endothelium-dependent vasodilation. The impairment of endothelium-dependent vasodilation in these patients is possibly not limited to the coronary circulation and may also be present in other vascular beds, a finding that could explain the evidence in previous studies (34) of the presence of a generalized disorder of vascular function in such patients.

Clinical implications. Abnormal coronary vasoconstrictor responses to acetylcholine have already been demonstrated in some groups of patients with angiographically normal coronary arteries (21,22). In patients with normal coronary arteries and hypercholesterolemia, acetylcholine infusion also induced a marked coronary vasoconstrictor response (22). In patients with atypical chest pain and normal coronary arteries, the vasomotor response to acetylcholine could be correlated with the presence of one or more coronary risk factors, of which hypercholesteremia, age >40 years and the presence of a family history of ischemic heart disease were most strongly correlated with an abnormal vasoconstrictor response to acetylcholine (21). The observation of a coronary vasoconstrictor response to acetylcholine therefore appears not to be specific for patients with angina and normal coronary arteries. However, in the present study, no significant correlation was found between any of the total number of coronary risk factors and the vasomotor response to acetylcholine in patients who had normal coronary arteries and either atypical chest pain or typical angina. These results are therefore apparently in contradiction to the study of Vita et al. (21). Their study (21), however, showed a broad scatter of data and an important variability within the same patient of different vessel segments exposed to the same risk factors. Furthermore, reexamination of their data after elimination of a low risk group (serum cholesterol level <200 mg/dl, age <40 years and total number of risk factors <2) showed that the correlation between the coronary vasomotor response to acetylcholine and any of the risk factors was also absent. From these considerations and the results of the present study, it appears that the coronary vasomotor response to acetylcholine cannot be predicted from the presence of one or more coronary risk factors in the individual patient or small patient groups. Pharmacodynamic testing with intracoronary administration of acetylcholine may therefore be useful in the diagnostic evaluation of patients with angiographically normal coronary arteries and

atypical or typical anginal chest pain because it may demonstrate the presence of a pathophysiologic substrate leading to abnormal coronary vasomotion that may contribute to the development of myocardial ischemia.

Finally, our study supports the recently introduced concept (35) of the role of distal coronary artery constriction in the pathogenesis of myocardial ischemia in some subsets of patients with ischemic heart disease. In nine patients in the group with coronary artery disease who developed angina during acetylcholine infusion, the left anterior descending artery was either smooth or showed only nonsignificant stenosis. The development of angina and myocardial ischemia in these patients during acetylcholine infusion into the left anterior descending artery therefore cannot be explained by paradoxic vasoconstriction and the resulting increase in flow resistance at the level of a coronary stenosis. Rather, as in the patients with angina and smooth coronary arteries, it must be attributed to the extensive nonocclusive coronary artery vasoconstriction caused by acetylcholine in the mid and distal segments of the left anterior descending artery. It therefore appears that pathologic constriction of distal coronary artery segments may represent an additional precipitating mechanism of myocardial ischemia in patients with coronary artery disease.

Conclusions. Endothelium-dependent vasodilation in response to acetylcholine is impaired in patients with normal coronary arteriograms and angina pectoris to a degree similar to that observed in patients with overt coronary artery atherosclerosis. The abnormal coronary vasoconstrictor responses resulting from this impairment may contribute to the pathogenesis of angina and myocardial ischemia in these patients.

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